

Attorney Docket No.: PENN-0754
Inventors: Scott L. Diamond
Serial No.: 09/763,982
Filing Date: April 25, 2001
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Amendments to the Specification:

Please replace the paragraphs beginning at page 17, line 1, with the following rewritten paragraphs:

--For purposes of chemical conjugation, the M9 sequence has been synthesized with a carboxy terminus addition of Gly-Gly-Gly-Cys (SEQ ID NO:19) to give an accessible thiol group provided by the cysteine:

Nuclear Targeting Peptide (NPS) -

NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY-GGGC (SEQ ID NO:1)

The K nuclear shuttling domain (KNS) of the hnRNP K protein is a 39 amino acid sequence:

YDRGRPGDRYDGMVGFSADETWDSAIDTWSPSEWQMAY (SEQ ID NO:4)

Example 5: Chemical conjugation of nuclear targeting epitopes to cationic scaffolds

Several different cationic scaffolds that are rich in amine suitable for conjugation reactions and which mediate electrostatic complexation or condensation with plasmid are used. These scaffolds can include: SV40 T antigen NLS (SVT = CGYGPKKKRKVGG (SEQ ID NO:5)), a mutated version of the SV40 T antigen NLS (muT = VKKGKCRPGKGYG (SEQ ID NO:2)), poly-L-lysine (MW 1, 4, and 30-70 kDa), histone H1, and hydrophilic amine-terminated dendrimers (87,340 MW) of small size (8.4 nm) available through Polysciences, Inc. (Warrington, PA). These scaffolds are tested for DNA condensation using a fluorescence quench assay of ethidium bromide labeled plasmid. Synthetic peptides with a C-terminus Gly-Gly-Gly-

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Cys-COOH (SEQ ID NO:19) linked to M9 or KNS are grafted at 1:1 to 3:1 to the activated amine-rich scaffolds. The crosslinker succinimidyl 4-(N-maleimidomethyl)cyclohexane-1 (SMCC, Pierce) is added at a final concentration of 10 mM (at 10% DMSO) to 100 µg amine rich scaffold (pH 7.2, 25°C for 2 hours) to react the SMCC NHS-ester to the primary amine of the scaffold. Excess SMCC and DMSO are removed by sephadex G-15 chromatography. The activated scaffold is conjugated with equimolar or 2 to 3-fold excess of the KNS or M9 peptide at 4°C (16 hours) by maleimide reaction with the SH moiety of the C-terminal cysteine. Individual reaction species are isolated by electroelution or FLPC. Precipitation with this method has not been observed.--